one modified 2'-deoxyfuranosyl moiety.

B2

18. The method of claim [1] 13 wherein at least one of the sugar linking groups of said modified oligonucleotide is replaced with a carbon or ether linkage.

REMARKS

Applicants affirm the election made by their attorney, John W. Caldwell, on April 25, 1991 of the species 2'-deoxyfuranosyl halogen and phosphorothioate substitutions and respectfully traverse the requirement for election. Applicants have found that some 2' substitutions of oligonucleotides confer nuclease resistance. It is respectfully pointed out, however, the invention is directed to the *location* of the substitution, rather than the *character* of the substitution. One skilled in the art would be able to determine a *number* of substitutions useful in conferring nuclease resistance to oligonucleotides of the present invention. In fact, Applicants have disclosed a number of such substitutions in their specification. This is equally true of the choice of backbone modification useful in the present invention. Therefore, Applicants should not be limited to only one such substitution and only one type of backbone modification. It is therefore respectfully requested that the requirement for restriction be withdrawn.

Claims 13-24 are pending in this case. Claims 13 and 18

have been amended. Claim 18 has been amended to correct an obvious typographical error.

Claims 13-24 have been rejected under 35 U.S.C. §112, first paragraph, because it was not demonstrated that Applicants' antisense oligonucleotides have an effect on modulating protein production in an intact organism. Applicants respectfully traverse this rejection. In an earnest effort to advance the prosecution of this application, Claim 13 has been amended to provide a method of modulating protein by contacting 2'-substituted oligonucleotides or oligonucleotide analogs with RNA or DNA coding for said protein. This amendment reflects that the invention is directed toward *the modulation of the production of protein* by contacting 2'substituted oligonucleotides with RNA or DNA coding for a protein. Support for this amendment can be found, for example, at page 8, line 35 through page 9, line 2 and lines 15-20 and throughout the specification.

Antisense methodology is the complementary hybridization of relatively short oligonucleotides to single stranded mRNA or single stranded DNA such that the normal, essential functions of these intracellular nucleic acids are disrupted (page 2, lines 3-7). A serious deficiency of oligonucleotide methodology has been the enzymatic degradation of the administered oligonucleotide by a variety of nucleases (page 3, lines 9-18). Applicants have provided an antisense methodology which differs from methods known

in the art in that the administered oligonucleotides are 2'-substituted oligonucleotides which hybridize with fidelity *and are nuclease resistant*.

Applicants provide a method for modulating protein production with *2'-substituted* oligonucleotides. Applicants have shown that these 2'-substituted oligonucleotides are nuclease resistant by Examples set forth in the specification at page 36, line 19 through page 37, line 23. It is shown that 2'-modified oligonucleotides demonstrate increased resistance to serum and cytoplasmic nucleases compared to natural phosphorothioate oligonucleotides. In addition, Applicants' 2'-modified oligonucleotides exhibited increased resistance to specific endo- and exo- nucleases, as compared to natural oligonucleotides. The utility of Applicants' improved antisense methodology should not depend upon showing of proof that antisense oligonucleotides do indeed modulate protein production. This has been well established in the art. Applicants' antisense methodology involves the administration of 2'-substituted oligonucleotides which are *nuclease resistant* to enhance the already well known method of antisense modulation of protein production. In light of the above, Applicants believe that no further showing of the modulation of protein production is necessary. Instead, Applicants have met the requirements of §112, first paragraph, by their showing of enhanced nuclease resistance of Applicants' improved oligonucleotides useful

in methods of the present invention. Therefore, Applicants respectfully request withdrawal of this §112 rejection.

Claims 13-24 have been rejected under 35 U.S.C. §112, second paragraph, because Applicants have not specified the type of organism for which their invention would be useful. Amended claim 13 provides a method of modulating protein by contacting 2'-substituted oligonucleotides or oligonucleotide analogs with RNA or DNA coding for said protein. The invention as presently claimed is not dependent upon the environment in which the modulation occurs, be it an organism or a petri dish. Therefore, Applicants believe that claims 13-24, in light of amended claim 13, satisfy 35 U.S.C. §112.

Claims 13-24 have been rejected under 35 U.S.C. §103 as being unpatentable over Ikehara in view of Marcus-Sekura.

Ikehara et al. synthesized poly (2'-chloro-2'-deoxyadenylic acid) and poly (2'-bromo-2'-deoxyadenylic acid) and found that the 2'-substituents exert a significant effect on thermal stability of the polynucleotides. It was found that 2'-substituents had a destabilizing effect on the thermal stability of the molecule when complexed with other polynucleotide strands, thus lowering the melting temperature (Tm) of the molecules. The melting temperature (Tm) is the temperature at which double or triple stranded nucleic acid molecules denature into separate strands. The melting temperature (Tm) of a molecule is also a

measure of relative binding affinity between nucleic acid strands, and in this case between the polynucleotide and a target molecule. Thus, oligonucleotides having a high melting temperature (Tm) bind with greater affinity to a target molecule than do oligonucleotides having a lower melting temperature (Tm). In particular, Ikehara et al. found that the Cl, Br and N_3 substituents at the 2' position *significantly lowered* the thermal stabilities of the double helix complexes at page 4259. The polynucleotides were also determined to be resistant wholly to ribonuclease M^{11} and partly to snake venom phosphodiesterase.

Marcus-Sekura et al. compared several classes of oligonucleotides for their ability to inhibit synthesis of chloramphenicol acetyl transferase (CAT). They recognized that normal oligonucleotides have relatively short half-lives due to nuclease degradation. Marcus-Sekura et al. suggested that replacement or modification of the oligonucleotide sugar-phosphate backbone by or to a phosphorothioate or methylphosphonate can increase the oligonucleotide biological lifetime, enhance permeability into cells, and strengthen binding to polynucleotide targets under physiological conditions.

Claims of the present invention are directed to a method for modulating the production of a protein by contacting a selected sequence of RNA or DNA coding for the protein with an oligonucleotide or oligonucleotide analog having a sequence of

nucleotide bases specifically hybridizable with the selected sequence of RNA or DNA coding for the protein, and having at least one modified 2'-deoxyfuranosyl moiety. Modifications encompassed by the present invention include hydrogen, hydroxyl, halo, azido, amino, substituted amino, cyano, halomethyl, isocyanato, alkoxyl, thioalkoxyl, haloalkoxyl, alkyl sulfide, alkyl sulfonate, nitrate, nitrite, ammonium, allyloxy, or alkeneoxy. Oligonucleotides of the present invention may be further modified such that at least some of the sugar linking groups of said oligonucleotide are modified to comprise a phosphorothioate, methyl phosphonate, or phosphate alkylate. Applicants found that such sugar-modified oligonucleotides hybridized to the targeted RNA more strongly than did the unmodified type (Page 17, lines 6-7). In addition, Applicants found melting temperatures (Tm) increased with 2'-substituted adenosine diphosphates (Page 16, lines 15-16).

Obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination. *In re Fine*, 837 F.2d 1071 (Fed. Cir. 1988).

Ikehara et al. teaches away from the present invention by teaching that 2'-substituted polynucleotides lack thermal stability. Ikehara et al. states that while the 2'-substituents did not *inhibit* the formation of double or triple strand complexes, the thermal stability *was* affected. Thermal stability is

indicative of the stability of a duplex. A duplex with a high melting temperature (Tm) is more stable than a duplex with a low melting temperature (Tm), *at any temperature*. Thus, Ikehara et al. teaches that a feature integral to effective antisense therapy, i.e., binding affinity, is compromised by the addition of the 2'-substituents.

Further, it has been shown that 2'-polynucleotide sequences, which Ikehara et al. asserts exhibit nuclease resistance on page 4253, have been found instead to lack nuclease resistance. See Kawasaki et al., *Synthesis and Biophysical Studies of 2'dRIBO-F Modified Oligonucleotides*, Conference on Nucleic Acid Therapeutics, Clearwater, FL (1/13-16/1991), a copy of which is attached hereto for the Examiner's convenience. Thus, contrary to the assertion by Ikehara et al. that any and all 2'-substituted oligonucleotides have nuclease resistance, just the opposite has been found to be true. Further, there is no suggestion or incentive in Ikehara et al. to use oligonucleotides set forth in Ikehara et al. for modulating the production of protein by cells. Marcus-Sekura et al. provides no incentive or suggestion to modify the production of protein using *2'-modified* oligonucleotides. Without such suggestion or incentive, Marcus-Sekura et al. alone, or in combination with Ikehara et al. does not make the present invention obvious. Therefore, Applicants respectfully request withdrawal of this §103 rejection.

In light of the above, Applicants believe that the application is in a condition ready for allowance. Therefore, Applicants respectfully request an early and favorable Notice of Allowance.

Respectfully submitted,

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